

Short- and long-term importance of small sharks in the diet of the rare deep-sea shark *Dalatias licha*

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Abstract Knowing the trophic ecology of marine predators is essential to develop an understanding of their ecological role in ecosystems. Research conducted on deep-sea and threatened shark species is limited. Here, by combining analyses of individual stomach contents and stable isotope values, we examined the trophic ecology (dietary composition and trophic position) of the kitefin shark *Dalatias licha*, a deep-sea shark considered as near threatened globally and as data deficient in the Mediterranean Sea. Results revealed the importance of small sharks in the diet of the kitefin shark at short- and long-term scales, although finfish, crustaceans and cephalopods were also found. Predation on sharks reveals the high trophic position of the kitefin shark within the food web of the western Mediterranean Sea. Stable isotope values from liver and muscle tissues confirmed our results from stomach content analysis and the high trophic position.

Introduction

Determining the feeding ecology of a particular organism is essential to understanding its ecological role in the ecosystem. Sharks are often classified as top predators within

marine ecosystems, although there are important differences in their diets between species (Cortes 1999). In fact, the diversity of feeding strategies complicates the understanding of the ecology of this marine group (Cortes 1999). As predators exerting top-down influences on communities coupled with declines in populations, sharks have become the focus of recent marine ecology research (Myers et al. 2007; Field et al. 2009; Ferretti et al. 2010). However, the trophic role that individual species play within marine communities in many ecosystems is still often unclear, which precludes the prediction of the consequences of their removal. To unravel this problem, more studies of species-specific trophic characteristics are essential, as these can inform conservation strategies for vulnerable or threatened species (Ferretti et al. 2013).

The Mediterranean Sea supports a relatively rich fauna of elasmobranchs and is considered a global hotspot of threatened elasmobranchs (Malak et al. 2011; Dulvy et al. 2014). However, this sea has been described as the most dangerous sea for these marine predators (Malak et al. 2011), due to diverse, interacting and intense threats from human activities (Coll et al. 2012, 2014). Although direct fisheries targeting sharks have caused stock collapses in some species, the major threats to Mediterranean chondrichthyans are the indirect impact of mixed fisheries and bycatch (Malak et al. 2011). As a consequence, around 40 % of the Mediterranean chondrichthyan species are considered threatened by the International Union for the Conservation of Nature (IUCN) (and they are classified as either Critically Endangered, Endangered, Vulnerable or data deficient; Malak et al. 2011).

In comparison with the more abundant shark species that inhabit continental shelf and slope areas, research focusing on sharks inhabiting the deep Mediterranean Sea is very limited (Malak et al. 2011), even though they potentially

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play an important ecological role in the dynamics of the ecosystem (Tecchio et al. 2013). This is the case of the kitefin shark *Dalatias licha*, a deep-sea shark globally considered as near threatened by the IUCN and as data deficient (DD) in the Mediterranean Sea (Blasdale et al. 2009). The kitefin shark is a marine predator distributed worldwide across warm, tropical and temperate oceans (Blasdale et al. 2009; Froese and Pauly 2013). In the Mediterranean, it is present mainly in the western basin, but also occurs in the eastern Levantine basin and the south-western Mediterranean (Blasdale et al. 2009; Bradai et al. 2012; Froese and Pauly 2013).

The deep-sea preferences of kitefin shark probably explain the limited number of studies on this species in the Mediterranean Sea (i.e. Macpherson 1980; Matallanas 1982; Kabasakal and Kabasakal 2002; Capapé et al. 2008). For example, in the western Mediterranean, since the work of Macpherson (1980) and Matallanas (1982) more than 30 years ago, there has been no new information on the trophic ecology of the kitefin shark. These two past studies indicated that the diet of the kitefin shark was composed mainly by demersal fin-fishes, cephalopods, small demersal sharks and crustaceans (Macpherson 1980; Matallanas 1982). The kitefin shark has also been reported to feed on epipelagic fast-swimming fishes such as the Atlantic bonito (*Sarda sarda*), and often, chunks of large fish are found in its stomachs, which may indicate scavenging or ambushing activity (Matallanas 1982). During recent decades, the Mediterranean ecosystem has been dramatically transformed as a consequence of an increase in fishing pressure and other human impacts such as pollution and global warming (Coll et al. 2012; Micheli et al. 2013). Therefore, the composition of species in the area has been modified, and this has probably had an impact on the prey availability and thus feeding ecology of the kitefin shark.

The study of feeding ecology of marine predators has traditionally relied on stomach content sampling, which captures short-term diet (usually <1 day for an individual, Hyslop 1980). Although such data permit high levels of taxonomic resolution, sharks often have empty stomachs and the preys that are recovered are often skewed towards those that are difficult to digest (Hyslop 1980). Moreover, stomach content analyses generally require large sample sizes to accurately quantify long-term feeding patterns (Hyslop 1980; Cortes 1999), which are difficult to obtain for most species of sharks, particularly those threatened or endangered (Stergiou and Karpouzi 2001; MacNeil et al. 2005). The use of stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) has been used as complementary tools to study feeding ecology of predators during the last decades (Kelly 2000; Ramos and González-Solís 2012; Shiffman et al. 2012). This approach is based on the fact that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values are transformed from dietary sources

to consumers in a predictable manner (Kelly 2000). Moreover, by combining stable isotope values for consumers with those from their potential prey, isotopic mixing models can be applied to obtain estimates of the relative contribution of each prey item to the diet of the consumer (e.g. by using the Stable Isotope Analysis in R [SIAR] isotopic mixing model, Parnell et al. 2010). By analysing the stable isotopic values in different tissues with different turnover rates, one can also determine the main diet of a particular organism on different timescales (Hussey et al. 2010; Shiffman et al. 2012). Although outcomes of stomach content analysis and isotopic mixing models should be interpreted with caution, their combination is valuable to a better understanding of the feeding ecology of organisms (Shiffman et al. 2012; Caut et al. 2013).

In the present study, we aimed to update and complement the available trophic information (dietary habits and trophic position) of the kitefin shark in the western Mediterranean Sea (Fig. 1). Specifically, by combining the use of stomach content and stable isotopic approaches, we aimed to examine the feeding ecology of this deep-sea shark at different temporal scales: in a short-term time frame (~1–7 days) by using stomach content analysis and long-term dietary information (~1 month for liver and ~1 year for muscle; MacNeil et al. 2005; Logan and Lutcavage 2010; Caut et al. 2013) by using the stable isotope approach. We evaluated the effect of sex (males and females) and area (Gulf of Lions and Catalan Sea, Fig. 1) on the feeding strategies of the kitefin shark. Our study provides new insights into the ways in which the kitefin shark exploits trophic resources and contributes to an understanding of its ecological role within the community.

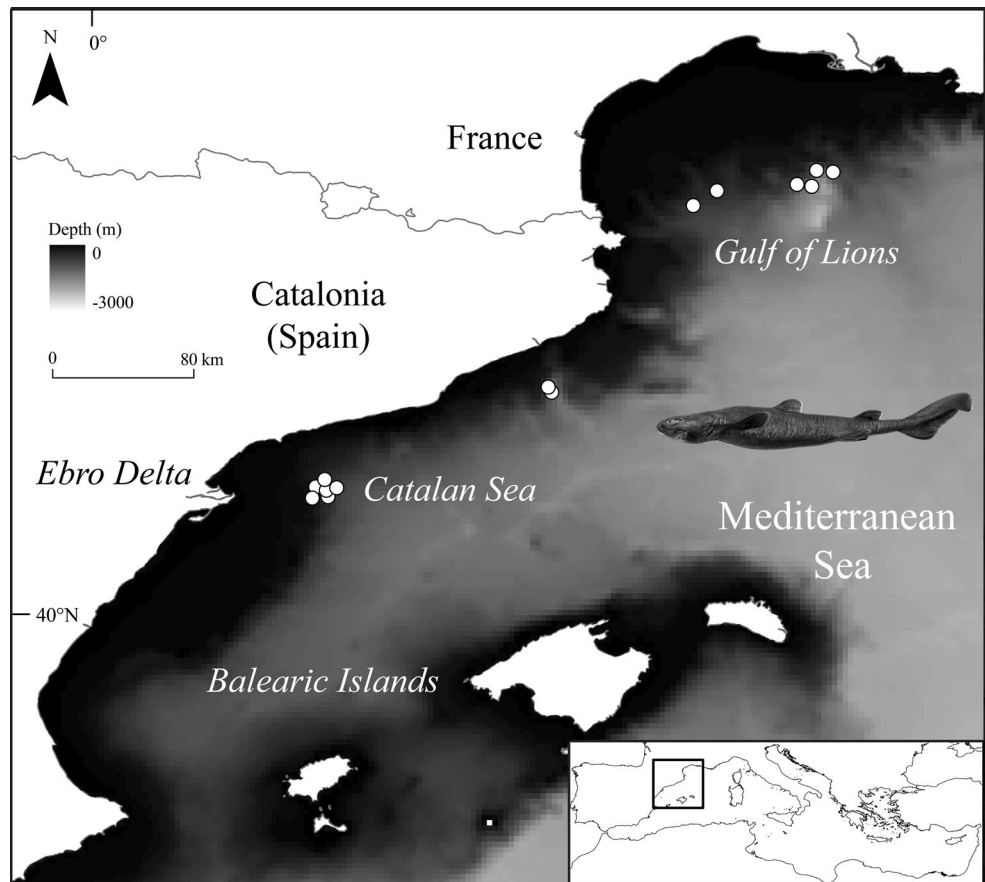
Materials and methods

Study area and sampling procedure

This study was conducted in the western Mediterranean Sea (Catalan Sea and Gulf of Lions, Fig. 1). The Catalan Sea is a highly productive marine area due to the combination of the Ebro River run-off and the effect of the Liguro-Provençal-Catalan current along the continental slope (Estrada 1996; Salat 1996). Similar to the Catalan Sea, the Gulf of Lions is one of the richest and most productive areas in the western Mediterranean, as the Rhône River discharges nutrients and organic matter into the area. These are transported to the Catalan Sea by the cyclonic Northern Current and the Liguro-Provençal-Catalan front (Estrada 1996; Salat 1996).

We collected 36 kitefin shark individuals between 2011 and 2013 (13 in 2011, 18 in 2012 and 5 in 2013) though all the year (winter, spring, summer and autumn); 32

Fig. 1 Map of the study area (north-western Mediterranean), indicating the sampling locations (white circles). A picture of kitefin shark *Dalatias licha* is also displayed (L. López)



specimens were accidentally collected as bycatch by the bottom trawling fleet working in the Gulf of Lions and in the Catalan Sea, and four specimens were captured during an experimental oceanographic bottom trawling cruise in the area (Fig. 1). The depths of the captures ranged between 350 and 550 m in the Gulf of Lions and 400–1,200 m depth in the Catalan Sea. Each specimen was immediately frozen on board after capture and stored at -20°C until their morphology; stomach content and tissue isotopic analyses were conducted.

The sex, stretch total length (in cm) and body mass (in g) of each specimen were recorded. We also recorded the maturity state as immature or mature of each individual, following the protocol by Stehmann (2002). In the case of males, the maturity state was determined according to the degree of calcification of the clasper and the degree of development of the testes and reproductive ducts. In the case of females, the maturity state was determined by the condition of uteri, oviducal glands and ovarian follicles (Stehmann 2002).

Stomach content analysis

Stomach contents were extracted after dissection. Each stomach was weighed on a digital balance and its contents

extracted. Each prey found in the stomach was weighed and identified to the lowest taxonomic level possible. Whenever fragments of prey were found, the number of counted individuals was the lowest as possible to avoid overestimation of the occurrence of a particular prey. To avoid potential biases associated with opportunistic trawl-net feeding, we only considered prey with evidence of digestion and removed all prey found in the teeth of the studied specimens when performing stomach content analysis. This potential problem is solved by using stable isotopic approaches within a comparative approach because N and C isotopic values only inform on the ingested food previously to the capture of the specimens (around 1 month or several months for liver and muscle, respectively).

To assess the importance of different prey in the diet, the combined Index of Relative Importance (Pinkas et al. 1971) was used as follows:

$$\text{IRI}_i = (N_i + W_i) \cdot \text{FO}_i \quad (1)$$

where FO_i = frequency of occurrence of a type of prey group (i) in relation to the total number of stomachs; N_i = contribution by number of a type of prey group (i) in relation to the whole content of the stomach; and W_i = wet mass of a type of prey group (i) in relation to the whole

content of the stomach. Unidentified prey was also included in the estimation of these trophic metrics.

Stable isotope analysis

A small portion of dorsal muscle (without skin or cartilage) and liver was extracted from each specimen. All muscle and liver samples were lyophilized after a lipid extraction technique was applied in the liver samples following Folch et al. (1957) to avoid for potential confusion in the interpretation associated with the high lipid concentration in the liver (Logan et al. 2008). All samples were subsequently freeze-dried and powdered, and 0.28–0.33 mg of each sample was packed into tin capsules. Isotopic analyses were performed at the Laboratory of Stable Isotopes of the Estación Biológica de Doñana (www.ebd.csic.es/lie/index.html). Samples were combusted at 1,020 °C using a continuous flow isotope ratio mass spectrometry system (Thermo Electron) by means of a Flash HT Plus elemental analyser interfaced with a Delta V Advantage mass spectrometer which applies international standards, run each 9 samples; LIE-CV and LIE-PA, previously normalized with the international standards IAEA-CH-3, IAEA-CH-6, IAEA-N-1 and IAEA-N-2. Stable isotope ratios were expressed in the standard δ -notation (‰) relative to Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric N_2 ($\delta^{15}\text{N}$). Based on laboratory standards, the measurement error (standard deviation) was ± 0.1 and ± 0.2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Urea was not removed from the tissues. The C:N ratio of all tissues was always lower than 3.5 ‰, and hence, no correction of the $\delta^{13}\text{C}$ values was required to account for the presence of lipids in muscle samples (Logan et al. 2008).

Isotopic mixing models and isotopic niche

We applied the SIAR Bayesian isotopic mixing model (Stable Isotope Analysis in R, SIAR 4.1.3; Parnell et al. 2010) to estimate the diet composition of kitefin sharks based on their muscle and liver isotopic values and those of their potential prey (small sharks, fin-fish, crustaceans and cephalopods). This model runs under the free software R (R Development Core Team 2009) and allows the inclusion of sources of uncertainty in the data, in particular the variability in the stable isotope ratios of the predator and the potential prey (Parnell et al. 2010). SIAR uses Markov-chain Monte Carlo modelling and fits a Bayesian model of the diet habits based on a Gaussian likelihood function (Parnell et al. 2010). The model also assumes that each target value (i.e. the stable isotope ratios for each individual) comes from a Gaussian distribution with an unknown mean and standard deviation. The mean was weighted by the isotopic values of each food sources. The standard deviation

depends on the uncertainty in the fractionation corrections and the natural variability among target individuals within a defined group (Shiffman et al. 2012). As we did not find significant differences in the stable isotopic values between sexes and sampling areas (see Results section), we considered all individuals together.

To build the SIAR mixing model, we used published reference values for potential prey groups of the kitefin shark from the western Mediterranean (sharks: $\delta^{15}\text{N} = 8.7 \pm 0.8$, $\delta^{13}\text{C} = -18.4 \pm 0.6$; fin-fish: $\delta^{15}\text{N} = 8.6 \pm 0.9$, $\delta^{13}\text{C} = -18.4 \pm 0.6$, crustacean: $\delta^{15}\text{N} = 7.1 \pm 1.5$, $\delta^{13}\text{C} = -19.2 \pm 0.9$, cephalopod: $\delta^{15}\text{N} = 7.5 \pm 0.8$, $\delta^{13}\text{C} = -19.0 \pm 0.6$; Albo-Puigserver et al. submitted for publication). Crustaceans, fin-fish and cephalopods were collected in the stomachs of the kitefin shark without evidence of digestion. In the case of small shark prey (the blackmouth catshark *Galeus melastomus* and the velvet belly lantern-shark *Etmopterus spinax*), we used stable isotopic values of specimens collected in the same area of kitefin shark by bottom trawling (Albo-Puigserver et al. submitted for publication). We used different isotopic discrimination factors for muscle ($\Delta\delta^{15}\text{N} = 1.95 \pm 0.26$ ‰, $\Delta\delta^{13}\text{C} = 0.49 \pm 0.32$ ‰; Hussey et al. 2010) and liver ($\Delta\delta^{15}\text{N} = 1.50 \pm 0.54$ ‰, $\Delta\delta^{13}\text{C} = 0.22 \pm 1.18$ ‰; Hussey et al. 2010). As a measure of trophic width, we calculated the Bayesian isotopic ellipse area (SEA) for each tissue (Jackson et al. 2011). This metric represents a measure of the total amount of isotopic niche exploited by a particular depredator and is thus a proxy for the extent of trophic diversity (or trophic width) exploited by the species considered (high values of isotopic standard ellipse areas indicate high trophic width). This metric uses multivariate ellipse-based Bayesian metrics. Bayesian inference techniques allow for robust statistical comparisons between data sets with different sample sizes. Isotopic standard ellipse areas were calculated using the routine Stable Isotope Bayesian Ellipses (SIBER, Jackson et al. 2011) also incorporated in the SIAR library.

Trophic position

We estimated the trophic position (TP) of each species by using stomach content information ($\text{TP}_{\text{stomach}}$) and stable isotopic analysis (TP_{liver} and $\text{TP}_{\text{muscle}}$).

With the stomach content, we calculated the $\text{TP}_{\text{stomach}}$ of each species using the following equation:

$$\text{TP}_j = 1 + \sum_{i=1}^n \text{DC}_{ji} \cdot \text{TP}_i \quad (2)$$

where j is the predator of prey i , DC_{ji} is the fraction of prey i in the diet of predator j and TP_i is the trophic position of prey i . The TP_i used were obtained from previous food web modelling studies conducted in the western Mediterranean

Table 1 Mean and SD of stretch total length, body mass and stable isotopic values in liver and muscle of the kitefin shark *Dalatias licha* $N = 36$ individuals

	Gulf of Lions		Catalan Sea	
	Males ($n = 9$)	Females ($n = 9$)	Males ($n = 8$)	Females ($n = 9$)
Stretch total length (m)	0.37 ± 0.05	0.36 ± 0.03	0.58 ± 0.27	0.54 ± 0.27
Body mass (kg)	0.22 ± 0.12	0.19 ± 0.51	1.76 ± 1.89	1.65 ± 2.83
Liver- $\delta^{15}\text{N}$ (‰)	10.22 ± 0.51	9.86 ± 0.59	10.11 ± 0.89	10.28 ± 0.74
Liver- $\delta^{13}\text{C}$ (‰)	-18.42 ± 1.76	-18.31 ± 1.36	-18.26 ± 1.97	-17.64 ± 1.81
Muscle- $\delta^{15}\text{N}$ (‰)	10.11 ± 0.51	10.02 ± 0.52	10.71 ± 0.69	10.17 ± 0.44
Muscle- $\delta^{13}\text{C}$ (‰)	-18.45 ± 0.51	-18.38 ± 0.79	-18.56 ± 1.72	-18.13 ± 0.44

area ($\text{TP}_{\text{fish}} = 3.05$, $\text{TP}_{\text{shrimps}} = 2.57$; $\text{TP}_{\text{crabs}} = 2.53$; $\text{TP}_{\text{cephalopods}} = 3.63$; Coll et al. 2006; Bănară et al. 2013).

We estimated the TP_{liver} and $\text{TP}_{\text{muscle}}$ of kitefin sharks based on isotopic values for each species according to the algorithm proposed by Vander Zanden and Rasmussen (2001):

$$\text{TP}_{\text{consumers}} = \text{TP}_{\text{basal}} + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{basal}}) / \Delta\delta^{15}\text{N} \quad (3)$$

where $\delta^{15}\text{N}_{\text{consumer}}$ and $\delta^{15}\text{N}_{\text{basal}}$ were, respectively, the $\delta^{15}\text{N}$ values of kitefin shark and the $\delta^{15}\text{N}$ values of krill (*Meganyctiphanes* sp.) in the western Mediterranean (Cardona et al. 2012). For the $\Delta\delta^{15}\text{N}$ value, we used the discrimination factors provided by Hussey et al. (2010) for muscle and liver (see previous subsection).

Statistical analysis

Differences in stretch total length, body mass, %W, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ between sexes and sampling areas (Catalan Sea and Gulf of Lions) were tested by using two-way semi-parametric permutation multivariate analyses of variance tests (PERMANOVA tests) on the Euclidean distance matrix (Anderson et al. 2008). Although the number of mature specimens in both areas was very low, we also tested the difference in diet between mature and immature individuals. Since we did not find any dietary significant results (PERMANOVA tests, for all cases $p > 0.05$), we have excluded this comparison from the study. This exclusion could be a bias which could have been evaluated if the sample size had larger. In the case of a significant result, pairwise tests were performed. PERMANOVA allows for the analysis of complex designs (multiple factors and their interaction) without the constraints of multivariate normality, homoscedasticity and having a greater number of variables than sampling units of traditional ANOVA tests. The method calculates a pseudo-F-statistic directly analogous to the traditional F -statistic for multifactorial univariate ANOVA models, using permutation procedures to obtain p values for each term in the model (Anderson et al. 2008). PERMANOVA tests were carried out with PRIMER-E 6 software (Anderson et al. 2008). The significance level for all tests was adopted at $p < 0.05$.

Table 2 Summary of PERMANOVA (n permutations = 999) test results examining differences between sexes and zones (Gulf of Lions and Catalan Coast) in body mass, stretch total length, stomach content (%W) and isotopic values of liver and muscle of *Dalatias licha*

Parameter	Factor	Pseudo-F	P (perm)
Body mass	Zone	8.72	0.003
	Sex	0.14	0.71
	Sex*zone	0.006	0.78
Stretch total length	Zone	0.01	0.002
	Sex	0.15	0.71
	Sex*zone	0.005	0.31
Stomach content	Zone	1.87	0.11
	Sex	1.24	0.23
	Sex*zone	2.37	0.07
Liver- $\delta^{15}\text{N}$	Zone	0.43	0.49
	Sex	0.16	0.66
	Sex*zone	1.29	0.25
Liver- $\delta^{13}\text{C}$	Zone	0.49	0.47
	Sex	0.39	0.54
	Sex*zone	0.19	0.66
Muscle- $\delta^{15}\text{N}$	Zone	3.27	0.06
	Sex	2.89	0.10
	Sex*zone	1.54	0.21
Muscle- $\delta^{13}\text{C}$	Zone	0.004	0.86
	Sex	0.54	0.48
	Sex*zone	0.29	0.61

$N = 36$ individuals

* Statistical significance < 0.05

Results

The total sample was composed of 36 kitefin shark individuals (18 females and 18 males), in which 30 were immature and 6 were mature. From the 36 individuals, 17 came from the Catalan Sea and 19 from the Gulf of Lions (Fig. 1). The body mass and stretch total length of kitefin sharks were similar between sexes but differed significantly between areas (Table 1 and 2). In particular, kitefin sharks collected in the Gulf of Lions were smaller in mass and length to the individuals collected in the Catalan Sea (Tables 1, 2).

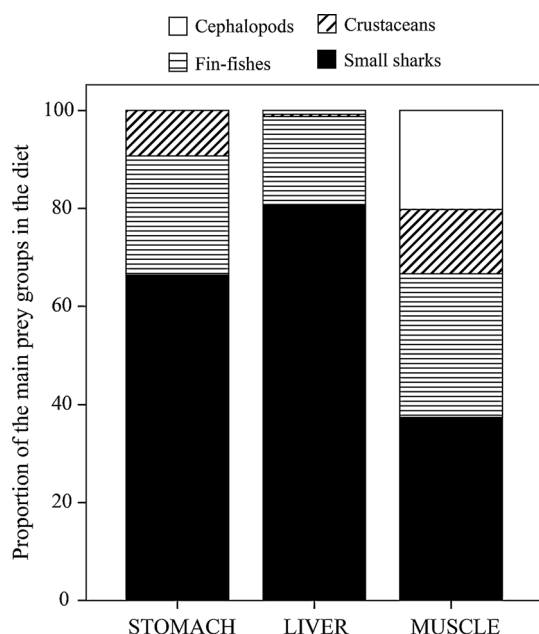


Fig. 2 Mean proportional contribution of different potential prey types to the diets of *Dalatias licha* in the western Mediterranean sea based on stomach content (in %W), liver and muscle isotopes (SIAR model)

Stomach content analysis

Based on the %W, the diet composition of kitefin shark did not differ between sexes or sampling areas (Table 1, 2; Fig. 2). Taking into consideration all specimens, stomach content results indicated that the diet of kitefin sharks included mainly small demersal sharks such as velvet belly lanternshark and blackmouth catshark (Tables 3, 4; Fig. 2), followed by fin-fishes (such as the Carapidae *Echiodon dentatus* and Mediterranean codling *Lepidion lepidion*, Tables 3, 4; Fig. 2) and crustaceans (such as the shrimps *Pasiphaea* sp. and Norway lobster *Nephrops norvegicus*, Tables 3, 4; Fig. 2). Other prey groups such as a passerine bird, polychaetes, foraminifers and tunicates were also found in the stomach but with a very low frequency and importance (based on %W or %IRI).

Isotopic results and SIAR model

$\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values of muscle and liver did not differ between sexes and sampling areas (Tables 1, 2). The feasible contribution of each potential prey, estimated by the SIAR model, indicated that in the case of liver and muscle analysis, the importance of small demersal sharks in the diet of kitefin sharks was the highest, followed by fin-fishes and by crustaceans and cephalopods in a low proportion (Figs. 2, 3). Between tissues, the SIAR results from liver (Figs. 2, 3a) suggested that the proportion of small

Table 3 Stomach diet composition of *Dalatias licha* in the western Mediterranean sea expressed as percentage frequency of occurrence (%FO), number (%N), mass (%W) and the index of relative importance (%IRI)

Prey item	%FO	%N	%W	%IRI
Foraminifers	10.00	12.31	0.01	7.49
Polychaetes	3.33	7.69	0.60	0.31
Crustaceans				
Decapoda <i>Pasiphaea</i> sp.	3.33	1.54	2.23	0.76
Decapoda <i>Nephrops norvegicus</i>	3.33	1.54	6.31	1.59
Natantia	16.67	12.31	0.17	12.64
Tunicate <i>Pyrosoma atlanticum</i>	3.33	7.69	0.60	1.68
Teleosts (fin-fishes)				
<i>Lepidion lepidion</i>	3.33	3.08	20.38	4.75
<i>Echiodon dentatus</i>	3.33	1.54	0.08	0.33
Unidentified teleosts	23.33	12.31	2.43	20.90
Elasmobranchs				
<i>Galeus melastomus</i>	6.67	3.08	28.15	12.65
<i>Etmopterus spinax</i>	13.33	9.23	26.85	29.24
Unidentified elasmobranch	10.00	4.62	7.35	7.27
Passerine bird unidentified	3.33	1.53	0.33	0.38

$N = 30$ individuals

demersal sharks in the diet (mean = 56 %) was higher than that as indicated by the SIAR results from muscle (mean = 38 %, Figs. 2, 3b). The importance of fin-fishes in the diet was similar between liver and muscle (Figs. 2, 3). On the contrary, liver results showed lower proportions of crustaceans (mean = 5 %) and cephalopods (mean = 9 %) than results from muscle tissue, which indicated on average a 12 and 20 % contribution for crustaceans and cephalopods, respectively (Figs. 2, 3).

Trophic position

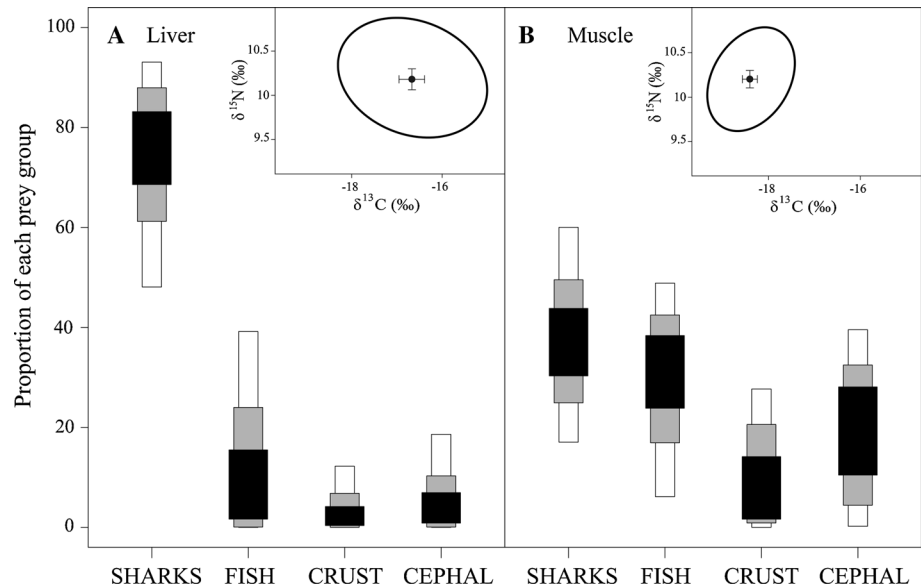
The trophic position estimated from stomach contents ($\text{TP}_{\text{stomach}}$) varied between 4.3 in the Catalan Sea and 4.7 in the Gulf of Lions. Although the trophic position estimated with stable isotopic N values of muscle (4.8 in the Catalan Coast and 4.6 in the Gulf of lions) was in the same range as the $\text{TP}_{\text{stomach}}$ values, estimates of trophic position estimated from liver tissues (5.5 in the Catalan Coast and 5.4 in the Gulf of Lions) were higher than the $\text{TP}_{\text{stomach}}$ and $\text{TP}_{\text{muscle}}$ in both areas.

Discussion

In this study, we present new information on the feeding ecology of a threatened Mediterranean chondrichthyan species, the kitefin shark (Blasdale et al. 2009). By combining

Table 4 Frequency of occurrence (%FO) and weight (%W) of the diet composition of *Dalatias licha* in the western Mediterranean of the present study and the values reported by Matallanas (1982) and Macpherson (1980) 30 years ago in the same area

	%FO			%W		
	Present study	Matallanas (1982)	Macpherson (1980)	Present study	Matallanas (1982)	Macpherson (1980)
Foraminifers	10.00	–	–	0.01	–	–
Polychaetes	3.33	2.50	–	0.00	0.01	–
Cephalopods	–	21.25	15.7	–	5.52	5
Crustaceans	20.00	32.50	41.8	9.19	7.04	14.6
Tunicates	3.33	–	–	0.60	–	–
Fin-fishes	26.67	72.50	73.4	24.12	71.26	60.3
Small sharks	30	23.75	20.9	65.71	14.68	14.3
Passerines	3.33	–	–	0.33	–	–

Fig. 3 Results of the SIAR model (95, 75 and 50 % credibility intervals) showing estimated prey contributions (SHARKS small sharks, FISH fin-fishes, CRUST crustaceans, CEPHAL cephalopods) of the diet of *Dalatias licha* in the western Mediterranean sea based on liver (a) and muscle (b) isotopic values. Mean and SE of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ and the standard ellipse areas of liver (upper-right corner of panel a) and muscle (upper-right corner of panel b) are also showed

the use of two complementary methodologies, stomach content and isotopic analyses, we have described the feeding ecology of this deep-sea shark at different temporal scales. In particular, stomach contents allowed the determination of the preys consumed in a short-time frame (~1–2 days), whereas the stable isotopic approach provided long-term dietary information (~1 month for liver and ~1 year for muscle; MacNeil et al. 2005; Logan and Lut-cavage 2010).

Our results revealed the consistent importance of small sharks in the diet of the kitefin shark, although demersal fin-fishes, crustaceans and cephalopods were also found to be part of its diet. In particular, both approaches revealed the high importance of blackmouth catshark and velvet belly lanternshark. Although previous studies recorded these small sharks in the diet of the kitefin shark based on stomach contents (Macpherson 1980; Matallanas 1982; Kabasakal and Kabasakal 2002; Capapé et al. 2008; Dunn et al. 2010, 2013), we provide strong evidence that this

feeding behaviour is consistent on short-term (stomach contents), medium-term (liver) and long-term (muscle) timescales, clearly indicating that this species can be considered a true shark predator (Munroe et al. 2013).

This feeding behaviour could be explained by two plausible complementary mechanisms: by nutritional demands related to prey-type or by interspecific trophic competition. Although it is well known that most demersal sharks have high lipid content in the liver, the kitefin shark is a species that, comparatively by size, presents extremely high lipid content in the liver probably to optimize its buoyancy in the deep-sea (Corner et al. 1969; Lewis 1969). For this reason, the kitefin shark could be consuming other sharks to obtain lipid resources from their livers. Complementarily, as both the blackmouth catshark and the velvet belly lanternshark coexist in the same habitat as the kitefin shark and exploit similar trophic resources (fin-fish and crustaceans; Albo-Puigserver et al. submitted for publication; Macpherson 1980; Fanelli et al., 2009), the kitefin shark could be

preying on them as a mechanism to reduce the number of potential competitors for food and space (Lourenço et al. 2013). From a general perspective, shark species that included other elasmobranches in their diet have been described worldwide (see review by Cortes 1999), but for Squaliformes species (the order of kitefin shark), this behaviour was only reported for 5 of the 32 species reviewed in Cortes (1999). Moreover, most of the shark species that consumed other sharks have a larger body size than the kitefin shark (Cortes 1999), which makes our results especially interesting. Despite our low sample size, we did not find a significant difference in the importance of shark preys in the diet of kitefin shark between mature and immature individuals. This could indicate that the importance of this prey resource is maintained throughout different life stages of the species.

The second prey group in importance in the diet of the kitefin shark was demersal fin-fishes. This prey group was cited as the main prey for kitefin shark in the western Mediterranean 30 years ago (Macpherson 1980; Matallanas 1982) (Table 4). Although not conclusive due to the reduced number of specimens analysed in the present study, this difference in the importance of fin-fish in the diet of the species could be due to a decrease in fish abundance in the western Mediterranean due to overfishing during the last decades (Coll et al. 2006, 2008; Cartes et al. 2013). Similar to previous studies conducted in the Mediterranean, both stomach and stable isotopic results indicated the low importance of the crustaceans in the kitefin shark's diet (Macpherson 1980; Matallanas 1982; Kabasakal and Kabasakal 2002; Capapé et al. 2008) (Table 4). In the Western Mediterranean, crustaceans probably are more important as food resource for elasmobranches present in shallower waters, where the availability of this resource is high, such as the case of the skates *Raja asterias* or *R. clavata*, or smaller demersal sharks such spotted dogfish *Scyliorhinus canicula* or velvet belly lantern shark (e.g. Valls et al. 2011; Navarro et al. 2013).

We found little evidence that cephalopods represented a major component of the diet of kitefin shark. This is notable as previous studies have mentioned cephalopods as an important prey for the kitefin shark (Macpherson 1980; Matallanas 1982) (Table 4) and other deep-sea sharks such bluntnose sixgill shark *Hexanchus griseus* or the Portuguese dogfish *Centroscymnus coelolepis* (Carrassón et al. 1992; Celona et al. 2005). Furthermore, the abundance of cephalopods has recently increased in the Mediterranean Sea (Coll et al. 2008, 2013a). The kitefin shark does not usually swallow prey whole, possibly explaining why no beaks, the typical indicator for cephalopod consumption, were found in the stomachs, underestimating their importance in the diet. However, stable isotopic results from liver and muscle also indicated that the importance of cephalopods for kitefin sharks was very low.

Surprisingly, we found a terrestrial bird (passerine) in the stomach of one kitefin shark. Although seabird specimens are mentioned as occasional prey for sharks (i.e. Simpfendorfer et al. 2001; Papastamatiou et al., 2006; Hallet and Daley 2011), this is the first evidence of a terrestrial bird as a prey for a deep-sea small shark in the Mediterranean. One explanation is that bird carcasses fall into the sea and become available for the kitefin shark. Knowing that large numbers of birds die in the Mediterranean during the migration between Europe and Africa (Newton 2008), we suggest that this finding could not be totally unexpected and perhaps could represent a seasonal resource pulse for the marine scavenger community (Bozzano and Sardà 2002; Fallows et al. 2013).

With the caveat of limited sample sizes, the diet composition of the kitefin shark was apparently independent of sex, sampling area and maturity state. The similar diet exhibited by male and female kitefin sharks can be explained by the fact that both sexes showed similar body mass and stretch total length (Wearmouth and Sims 2008, 2010). Sexual differences in diet are described for shark species with marked sexual dimorphism in size and/or feeding apparatus as a mechanism to reduce the intraspecific competition for food between males and females (Wearmouth and Sims 2008, 2010). Similarly, the diet composition was similar between Gulf of Lions and Catalan Sea, although we observed size differences, suggesting a spatial consistency in the feeding strategies of kitefin sharks, possibly because the abundance of their main prey is similar in both areas. The body size differences between both sampling areas could be directly related to the differences in depth between Gulf of Lions and the Catalan Sea. In particular, in the Catalan Sea, where the specimens were collected at deeper waters, the specimens of kitefin sharks were larger than the specimens collected in the Gulf of Lions. Size segregation by depth is a pattern recorded in deep-sea shark species (Kiraly et al. 2003; Veríssimo et al. 2003).

Knowing the trophic position of sharks is important to understanding their ecological position in relation to other organisms in the ecosystem (Cortes 1999; Stergiou and Karpouzi 2001). In this study, we calculated the trophic position of kitefin shark using both stomach content information and isotopic nitrogen values (Navarro et al. 2011; Mancinelli et al. 2013; Hussey et al. 2014). The trophic position calculated with the stomach results and with the $\delta^{15}\text{N}$ values of muscle was similar, indicating that the trophic position of kitefin shark in both the short- and long-term was around 4.6. In contrast, the trophic position estimated from the nitrogen values of liver was higher. These differences are related to the fact that in the mid-term (liver information), the importance of small sharks in the diet of kitefin shark was highest, increasing the estimated

trophic position in comparison with the stomach and muscle isotopic information. If we compare the trophic position calculated in our study with results from other studies, we observe that our results are slightly higher than other published data for this species (TP = 4.2 in Cortes 1999; TP = 4.35 ± 0.75 in Stergiou and Karpouzi 2001; TP = 4.6 in Dunn et al. 2013) or for other deep-sea sharks in the western Mediterranean (TP = 4.05 in Tecchio et al. 2013) likely due to the fact that these studies estimated the trophic position from diet studies where the importance of small sharks was lower (Macpherson 1980; Matallanas 1982).

The relative high trophic position of the kitefin shark indicates that this rare deep-sea predator is a potentially important predator of the Mediterranean food web (Coll et al. 2006; Bănară et al. 2013; Tecchio et al. 2013), but not necessarily in terms of the function in the ecosystem, which depends on the abundance, consumption and turnover of the species. Due to the likely very low current abundance of kitefin shark in the ecosystem, its ecological role may be potentially low or even negligible (Lotze et al. 2006).

In conclusion, we present new data regarding the feeding ecology and trophic position of the rare kitefin shark in the Mediterranean. Dietary analyses from two different methodological perspectives highlight the high importance of small demersal sharks in the diet of the kitefin shark. This points to the predatory role that this species plays in the ecosystem and the high trophic position that it occupies within the food web in the western Mediterranean Sea. The study emphasizes the utility of this combined approach for trophic studies due to its capacity for monitoring food web changes over different time spans. Data provided here for the kitefin shark allow further studies on the role that predatory species play in Mediterranean food webs (Piroddi et al. 2011; Ajemian and Powers 2013; Coll et al. 2013b).

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